inter-monomer coupling within the bc_1 dimer [4, 6] has remained elusive.

Here we have used large-scale molecular dynamics (MD) simulations for tracking the communications within the bc_1 of *Rhodobacter sphaeroides*. The energy correlation analysis [7] revealed the possible pathways for transmembrane propagation of information about the redox state of the ubiqinone molecule in the Q_N site. Non-equilibrium MD simulations of the bc_1 with ubiquinone or ubisemiquinone in the Q_N site, respectively, revealed the possible implication of helix *E* and the *ef*-loop of cytochrome *b* in the intra-monomeric transmembrane coupling. The MD simulations with occupied and unoccupied Q_P site showed a possible pathway for the inter-monomeric communication between the two Q_P sites of a bc_1 dimer.

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12P4

In vivo accumulation of coenzyme Q biosynthetic intermediates and aminated analogs in the yeast *Saccharomyces cerevisiae*

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Coenzyme Q (ubiquinone or Q) is a redox-active lipid essential for electron and proton transport in the mitochondrial respiratory chain. Q is also important in the mitochondrial inner membrane because it serves as an antioxidant and it modulates the function of the mitochondrial membrane transition pore [1]. Most Coq proteins which participate in Q biosynthesis are present in a high molecular mass multi-subunit complex in *Saccharomyces cerevisiae*. The absence of a single Coq polypeptide from the complex causes a drastic diminution of the steady state levels of some Coq proteins. In consequence, only an early intermediate of the Q biosynthetic pathway accumulates in Δ*coq1*–Δ*coq9* strains [2].

We report that overexpression of the protein kinase Coq8 restores the steady state levels of the Coq proteins in most Δ*coq* strains. The stabilization of the Coq polypeptides leads to the accumulation of Q biosynthetic intermediates [3]. These intermediates are likely not competent to transfer electrons in the respiratory chain because the strains are not capable of growing on non-fermentable carbon sources. However, we suggest that some of these Q biosynthetic intermediates have an antioxidant activity and are therefore able to redox-cycle *in vivo* since they diminish the sensitivity of the accumulating strains to poly-unsaturated fatty acids.

4-hydroxybenzoic acid is the precursor of the aromatic ring of Q. We demonstrate that analogs of 4-hydroxybenzoic acid can be used *in vivo* to either promote the synthesis of analogs of coenzyme Q [4] or to bypass a deficient Q biosynthetic step. In this later case, the use of vanillic acid allowed the restoration of Q biosynthesis and respiration in cells deficient for the monooxygenase Coq6 [5].

Our data illustrate the possibility to generate Q analogs *in vivo* which offers the opportunity to study the structural requirements of Q for its different cellular functions.

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12P5

How to inhibit bc_1 complex with antimycin A?

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Using a stochastic simulation without any other hypotheses, we have demonstrated the natural emergence of the Mitchell Q-cycle in the functioning of the bc_1 complex, with few short-circuits and a very low occupancy of the reactive semiquinone species in the Q_o site [1]. However, this simple model fails to explain both the inhibition by antimycin of the bc_1 complex and the accompanying increase in ROS production.

To obtain inhibition of electron transfer to the high potential chain in the presence of antimycin, it is necessary to block the electron transfer from the reduced haem b_L to the semiquinone in the Q_o site (short-circuit or bypass of type 2) [2]. Incorporating this constraint in our stochastic model, we obtain a sigmoid inhibition curve due to the fact that when only one antimycin is bound per bc_1 dimer, the electron of the inhibited monomer systematically crosses the dimer interface to reduce a quinone or a semiquinone species in the other (free) Q_i site (b_L–b_L path). Because this step is not limiting, the activity is unchanged (compared to the activity of the free dimer). Interestingly, this b_L–b_L pathway is almost exclusively taken in this half-bound antimycin dimmer. In the free dimer, the natural faster pathway is b_L–b_H on the same monomer (at least in the absence of $\Delta\mu H^+$). The addition of the assumption of half-of-the-sites reactivity to the previous hypothesis leads to a transient activation in the antimycin titration curve preceding a quasi-complete inhibition at antimycin saturation.

In accordance with the chemistry of quinone, we have examined the possibility that the return of the electron from the reduced haem b_L to the semiquinone in Q_o could be blocked if we take into account the protons transfer and release from the Q_o site accompanying the electron transfer. However preliminary simulations show that it is not

sufficient to completely block this bypass. We are currently trying to define more precisely the steps necessarily or possibly involved in the antimycin inhibition of bc_1 complex and their chemical/physical basis [3].

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12P6

The use of electron spin–lattice relaxation measurements in studying structural changes of iron–sulfur protein in cytochrome bc_1 from *Rhodobacter capsulatus*

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Cytochrome bc_1 (cyt bc_1) is a central enzyme of many respiratory and photosynthetic systems which couples the proton transport across the membrane with electron transfer from ubiquinone (Q) to cytochrome *c (*cyt *c)* pool. Cyt bc_1 from *Rhodobacter capsulatus* is composed of only three catalytically relevant subunits: cytochrome *b* (cyt *b*), cytochrome c_1 (cyt c_1) and iron–sulfur (FeS) protein. One of the unique features of the operation of cyt bc_1 is a large-scale movement of FeS head domain, which shuttles electrons between Q bound at Q-binding site (Q_o) at cyt *b* and cyt c_1. We employed the measurements of the electron longitudinal relaxation of the iron–sulfur cluster to analyze the efficiencies of the relaxation mechanisms associated with structural changes induced by mutations that lower the FeS midpoint potential (S158A or Y160W) and/or by the changes induced during the FeS movement. Low temperature dependence of the relaxation rates was measured by inversion-recovery at Q-band and analyzed assuming dominant direct and second-order Raman processes. In all cases the magnetization recovery was non single exponential with no signs of the contribution from spectral diffusion. We found a significant decrease in the efficiency of the Raman process (C_{ram}) in the order: Wild Type cyt bc_1 (WT)>Y160W>S158A which is in agreement with the theory that predicts a decrease of spin–phonon coupling with the decrease in g-anisotropy of the paramagnetic center. However the effect of additional mutations or the addition of inhibitors, that increase the FeS density at the Q_o site correlates with the decrease in the relaxation rates via Raman process what is manifested in lower C_{ram} values. At the same time there is no decrease in g-anisotropy which suggests that molecular flexibility is the factor that is responsible for modulations of the vibrations of the cluster buried in protein matrix. The lower C_{ram} values for samples with FeS in close contact with cyt *b* suggest that the increase in the structural rigidity of the protein environment around the cluster is caused by interaction of the FeS with other subunits. Observation that spin–lattice relaxation changes upon the shift of the FeS density at the Q_o provides an additional means of monitoring the FeS movement which is complementary to our previously reported method based on heme b_L — FeS spin–spin interactions independent on the redox state of hemes *b*.

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12P7

Human skin fibroblasts as a model of coenzyme Q_{10} deficiency

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Coenzyme Q_{10} is a lipophilic antioxidant and cell-growth regulator. It influences both proliferation and programmed cell death, but the main function is transferring electrons in mitochondrial respiratory chain and hence ATP production [1]. CoQ_{10} biosynthesis defects lead to CoQ_{10} concentration decrease and thus CoQ_{10} deficiency. This serious disorder has heterogeneous phenotype — mostly encephalopathy, myopathy or renal failure [2].

The aim of the study was characterization of primary CoQ_{10} deficiency *in vitro*. The model was created using 4-aminobenzoic acid (PABA) — a competitive inhibitor of polyprenyl-4-hydroxybenzoat transferase (Coq2p), which is a key enzyme in the CoQ_{10} biosynthesis [3].

Three primary cell cultures of human skin fibroblasts (HSF) acquired from healthy controls and HEK293 cell line were used as a model for this project.

Four-day incubation of HSF or HEK293 in cultivation medium (without FBS added) with 1 mM PABA caused significant decrease in CoQ_{10} concentration (60% or 40% resp. compared to control cells). The CoQ_{10}-deficient cells were viable. No changes in mitochondrial morphology and ultrastructure were observed.

Mitochondrial respiratory chain function was changed in the CoQ_{10}-deficient cells — the activity of NADH:cytochrome *c* reductase and succinate: cytochrome *c* reductase was decreased to 70% of control-cell activity. Production of reactive oxygen species (ROS) was increased (based on fluorescent staining, dihydroethidium). Addition of decylubiquinone to PABA-containing medium (6 μM, 24 h) increased activity of both enzymes significantly; the ROS production was decreased to control levels.

The CoQ_{10} deficiency model using primary cell cultures was established. This model is highly convenient for further CoQ_{10} deficiency studies, its diagnostics and therapy in human medicine.

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12P8

Inhibition of mitochondrial complex III by nitric oxide

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Nitric oxide (NO) plays central roles through its binding to the heme group of guanylate cyclase that produces cGMP, and it is able to